Editorial

Cell-Free Hemoglobin as an Oxygen Carrier Removes Nitric Oxide, Resulting in Defective Thromboregulation

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Development of a clinically safe and effective substitute for erythrocytes that is capable of efficient oxygen delivery in vivo has progressed to the stage where cross-linked hemoglobin preparations are now undergoing clinical trials.[1] Such preparations withstand storage for prolonged periods of time, can be administered without the need for cross-matching, and are free of contamination by infectious agents.

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Utilization of cell-free hemoglobin as an erythrocyte substitute was initially hampered by nephrotoxicity and an affinity for oxygen that prevented efficient oxygen delivery to tissues.[2] These disadvantages were overcome when Bunn and Jandl[3] chemically cross-linked the hemoglobin molecule to produce stable hemoglobin oligomers that do not pass through the glomerular filtrate. In addition, Benesch and Benesch[4] developed reagents that modified the 2,3-diphosphoglycerate binding site of hemoglobin, thereby reducing its oxygen affinity.

Administration of cell-free hemoglobin solutions results in systemic vasoconstriction in research animals.[5] This is thought to be a consequence of the high avidity of hemoglobin for nitric oxide (NO, endothelium-derived relaxing factor [EDRF]), which it binds and inactivates. The NO-hemoglobin interaction results in rapid formation of nitrite/nitrate and methemoglobin. This blocks vasodilation induced by NO via activation of vascular smooth muscle cell guanylate cyclase.[5-7]

Removal of NO by hemoglobin will also reduce activity of platelet guanylate cyclase. This increases platelet reactivity, resulting in platelet deposition on prothrombotic surfaces such as injured vessel wall. This phenomenon was indeed demonstrated by the experiments of Olsen et al[8] as reported in this issue of *Circulation*. Using a rat microsurgical carotid endarterectomy model, the authors showed that infusion of a cross-linked hemoglobin preparation ($\alpha\alpha$Hb) led to significant enhancement of platelet deposition on the injured blood vessel surface. This was due to the NO-scavenging property of $\alpha\alpha$Hb as demonstrated by the following observations: (1) Increased platelet deposition resulted from infusion of $\alpha\alpha$Hb as well as infusion of an inhibitor of NO synthase, N^G-monomethyl-L-arginine (NMMA), and (2) increased platelet deposition after $\alpha\alpha$Hb or NMMA administration was reversed by infusion of L-arginine, the precursor of NO. Thus, the data obtained in this model system document and emphasize the importance of NO as an endogenous thromboregulator.

Oral administration of aspirin failed to prevent the increase in platelet deposition induced by $\alpha\alpha$Hb infusion, although a small, beneficial effect cannot be excluded (Fig 2 in reference 8). Thus, the proaggregatory effect of cross-linked hemoglobin appears to occur via EDRF removal alone. Earlier, Broekman et al[9] had shown that EDRF/NO could block platelet reactivity in an aspirin-insensitive manner.

Thromboregulation

There is experimental evidence for at least three independent mechanisms in endothelial cells that act concurrently to downregulate platelet reactivity and defend against accumulation of an occlusive platelet-rich thrombus (the Figure). Loss of platelet reactivity in the presence of endothelial cells occurs via one or more of the following mechanisms (Table): (1) Formation of eicosanoids such as prostacyclin, either endogenously or via transcellular metabolism of released precursors from activated platelets[10]; (2) formation of EDRF/NO[9]; and (3) metabolism of prothrombotic, platelet-released ADP by endothelial cell ecto-ADPase.[11-13]

In the study by Olsen and colleagues,[8] prevention of cyclooxygenase-catalyzed eicosanoid formation by aspirin treatment did not protect against platelet deposition after infusion of $\alpha\alpha$Hb. Although not examined by Olsen et al, endothelial cell ecto-ADPase can completely inhibit platelet reactivity in vitro, even if cyclooxygenase-catalyzed eicosanoid formation and EDRF/NO production are blocked. The data of Olsen et al demonstrate conclusively that infusion of $\alpha\alpha$Hb results in destruction of EDRF/NO, thereby promoting platelet deposition at the site of experimental injury. Thus, infusion of $\alpha\alpha$Hb results in a breach in one of the components of the thromboregulatory system.

Results of these experiments demonstrate that a previously unappreciated property of hemoglobin, destruc-

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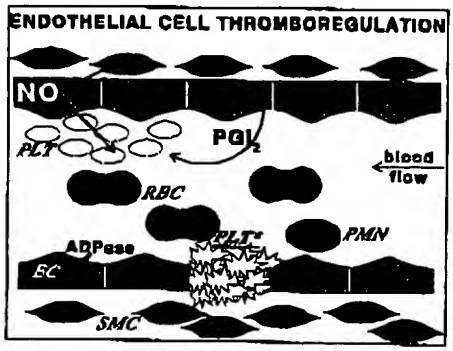

Diagram shows that when circulating platelets become activated, endothelial cells respond to limit or reverse the consequences of platelet adhesion, aggregation, and recruitment. We define this process as endothelial thromboregulation. In vitro, platelets become unresponsive to all agonists in the presence of endothelial cell suspensions. This is due to at least three separate thromboregulatory systems: (1) formation of eicosanoids from arachidonic acid; (2) generation of endothelium-dependent relaxing factor/nitric oxide (EDRF/NO) from arginine; and (3) ecto-nucleotidase(s) with both ADPase and ATPase activities.[13,14] Activation of endothelial cells by agonists such as thrombin results in formation of prostacyclin via cyclooxygenation of arachidonic acid. Prostacyclin reacts with a specific receptor on the platelet surface and initiates a G protein–linked signal transduction pathway, resulting in formation of cAMP. cAMP is a strong inhibitor of platelet function via antagonism of calcium-mediated platelet responses. EDRF/NO is an aspirin-insensitive fluid-phase autacoid produced by vascular endothelium and a variety of other cells and stimulates the soluble guanylyl cyclase in target cells. The resulting elevation in cGMP blocks responsiveness of activated platelets. In endothelial cells, NO is produced constitutively from L-arginine by a specific isoform of NO synthase.[15] The third endothelial thromboregulatory system involves ecto-nucleotidase(s) on the cell surface. These ecto-nucleotidase(s) are aspirin insensitive and metabolize released platelet ADP to AMP and adenosine, thereby limiting platelet recruitment.[11] EC indicates endothelial cell; PLT, platelet; PLT*, activated platelet; SMC, smooth muscle cell; RBC, erythrocyte; and PMN, neutrophil.

tion of EDRF/NO, can lead to a phenomenon with important clinical implications, ie, platelet deposition at sites of vascular injury, possibly leading to an aspirin-insensitive thrombotic diathesis.

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Human Endothelial Cell Thromboregulation

Type	Example	Fluid Phase	Aspirin-Sensitive	Function
Eicosanoids	Cyclooxygenase metabolites, PGI_2, PGD_2	Yes	Yes	Inhibit platelet reactivity by increasing cAMP
Nitrovasodilators	EDRF/NO	Yes	No	Inhibit platelet reactivity by increasing cGMP
Ecto-nucleotidases	ADPase(s)	No	No	Inhibit platelet reactivity by removing secreted ADP

PGI_2 indicates prostacyclin; PGD_2, prostaglandin D_2; EDRF, endothelium-derived relaxing factor; and NO, nitric oxide.